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Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves

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ABSTRACT

A comprehensive study on the volatile oil and phenolic constituents of *Ailanthus altissima* Swingle (Simaroubaceae) leaves was performed. Methanolic extracts of leaves and their hydrodistilled residues were screened for their antioxidant, antimicrobial and phytotoxic properties. The results showed that the leaf volatile oils were a complex mixture of more than a hundred components, mainly composed by non-terpenic compounds (tetradecanol, heneicosane, tricosane and docosane) and sesquiterpene hydrocarbons (α-curcumene and α-gurjunene). Methanolic extracts from leaves contain the highest level of total phenolic content, while those from the hydrodistilled residues showed the highest total flavonoid content. The most frequent phenolic compounds identified by HPLC-DAD–MS were gallic acid, chlorogenic acid, HHDP-galloylglucose, epicatechin, rutin, hyperoside and quercetin-3-galloyl hexoside. Evaluation of the antioxidant activities by using four complementary tests (DPPH, ABTS, 2-deoxyribose and FRAP) showed that both extracts exhibited strong concentration-dependent antioxidant activities. These extracts were efficient against Gram-positive bacteria, but not active against Gram-negative bacterial strains and the yeast *Candida albicans*. They also exhibited strong inhibitory effects on the germination and the radicle growth of the wild *Daucus carota*. This work provides scientific supports for the high antioxidant and phytotoxic activities of this species and thus, it may find potential applications in the development of natural herbicides and antioxidants for agro-food and pharmaceutical industries.

1. Introduction

Ailanthus altissima (Mill.) Swingle (Simaroubaceae), commonly known as the tree of heaven is a decidious species native to the northern and central mainland of China, Vietnam, Taiwan and Japan. Due to its ornamental characters, this species has been introduced and naturalized in many countries of the world (Kovarik and Säumel, 2007). The successful introduction of this species in a non-native range is attributed to its high growth rate, easy regeneration from soot suckers, high prolific seed (samaras) production, high tolerance to pollutants, great potential to compete for nutritive resources and its phytotoxic effects on neighboring plants (De Feo et al., 2003). It is a prominent multipurpose tree since it is used in indigenous medicine for the treatment of amoebic dysentery, colds and gastric diseases (De Feo et al., 2003; Singh et al., 2009). The antioxidant, antimalarial, antiviral, antifungal, insecticidal, antituberculosis, anti-inflammatory, cytotoxic, antiproliferative, antiasthmatic and phosphodiesterase inhibitory activities have previously been reported for *A. altissima* (Murakami et al., 2003; De Feo et al., 2005; Rahman et al., 2009; Lü and He, 2010; Rahimi et al., 2010; Luís et al., 2012). These biological activities were attributed to a plethora of bioactive components such as alkaloids, quassinoids, terpenoids, steroids, flavonoids and volatile oils, among others (Kundu and Laskar, 2010). Although that the chemical investigation of different *Ailanthus* species (i.e. *A. excelsa*, *A. vilmoriniana*, *A. malabarica* and *A. grandis*) have afforded nearly 200 compounds with varying structural patterns (Aono et al., 1994; Hung et al., 1995; Takeya et al., 1997; Loizzo et al., 2007), there is a dearth of information about the secondary metabolites of *A. altissima* and a comprehensive study on its chemistry is certainly missing. This knowledge, however, is crucial to define the possible application areas for the rational use of this species. Therefore, the main task of the present contribution was to: (*i*) assess the essential oil composition of *A. altissima* leaves, (*ii*) characterize their phenolic constituents and (*iii*) evaluate their antioxidant, antimicrobial and phytotoxic activities.

2. Materials and methods

2.1. Chemical and reagents

Folin-Ciocalteu, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxyribose, $FeCl_3$, 2,4,6-tripyridyl-S-triazine (TPTZ), potassium

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ferricyanide [$K_3Fe(CN)_6$] and trolox were purchased from Sigma-Aldrich Inc. (Steinheim, Germany). Solvents of analytical and HPLC grade were purchased from Carlo Erba Reactif-CDS (Val de Reuil, France). Thiobarbituric acid (TBA), trichloroacetic acid (TCA), HCl, ethylenediaminetetracetic acid (EDTA) and L-ascorbic acid were obtained from Merck (Darmstadt, Germany).

2.2. Plant material

Leaves of *A. altissima* were collected from healthy trees in three different localities from Tunisia: Jrissa (35°50′29″(N), 8°38′07″(E)); Bousalem (36°61′37″(N), 8°96′53″(E)) and Bab Saâdoun (36°80′85″(N), 10°15′92″(E)). Leaves were air dried at room temperature (20 ± 2 °C) for one week, ground using a Retsch blender mill (Normandie-Labo, Normandy, France), sifted through 0.5 mm mesh screen to obtain a uniform particle size and subsequently assessed for their essential oil composition.

2.3. Extraction and analysis of volatile oils

The air dried materials (100 g) were submitted to conventional hydrodistillation for 3 h by using a simple laboratory Quikfit apparatus that consisted of a 2000 mL distillation flask, a condenser and a receiving vessel. The obtained distillate was extracted twice with *n*-pentane (20 mL) and dried over anhydrous sodium sulfate (Hosni et al., 2008). The organic layer was then concentrated up to 1.5 mL at 35 °C using a Vigreux column and subsequently analyzed. The remaining residual leaf samples were dried, ground and stored for further uses.

Analytical gas chromatography was carried out on a Shimadzu HRGC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID), auto-injector AOC-20i and auto-sampler AOC-20s. Separation of volatile components was performed on an Rtx-1 capillary column. The oven temperature was programmed as follows: starting from 50 °C (10 min), increasing to 190 °C at a rate of 2 °C/min, then held for 10 min. The injector and detector temperature were programmed at 230 °C; the flow rate of the carrier gas (N_2) was 1.2 mL/min and the split ratio was 1:60.

The gas chromatography–mass spectrometry (GC–MS) analyses were performed on a gas chromatograph HP 6890 (II) interfaced with an HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, Ca, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (60 m × 0.25 mm, 0.25 mm film thickness) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 mL/min. Scan time and mass range were 1 s and 50–550 m/z, respectively.

The volatile compounds were identified by comparison of their retention indices relative to (C_7–C_{20}) *n*-alkanes, and by matching their mass spectral fragmentation patterns with corresponding data (Wiley 275.L library) and other published mass spectra (Adams, 2001), as well as by comparison of their retention indices with data from the Mass Spectral Library "Terpenoids and Related Constituents of Essential oils" (Dr. Detlev Hochmuth, Scientific consulting, Hamburg, Germany) using the MassFinder 3 software (www.massfinder.com). Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak areas without use of the correction factor.

2.4. Preparation of methanolic extracts

Dried and ground leaves and the residual leaf sample remained after distillation of *A. altissima* were mixed with methanol (1:20 w/v), sonicated (Transsonic type 310/H, Germany) for 30 min and macerated for 24 h. The macerated methanolic extracts were filtered through Wattman #1 filter paper (Bärenstein, Germany), centrifuged (Eppendorf 5810R, Le Pecq, France) for 10 min at 3000 *g* and concentrated under reduced pressure in a Heidolph rotary evaporator (Schwabach, Germany).

2.5. Phytochemical analysis

2.5.1. Total phenolic (TP) content

Total phenolics were determined with the Folin-Ciocalteu assay according to the procedure reported by McDonald et al. (2001). Briefly, 500 μL of appropriately diluted extract was added to 5 mL of freshly diluted 10-fold Folin-Ciocalteu reagent, and the mixture was neutralized with 4 mL of a sodium carbonate solution (7%). The reaction mixture was kept in the dark for 15 min, and its absorbance was measured at 765 nm against a blank, which was prepared according to the procedure described above except that the extract solution was substituted by 500 μL of distilled water. Gallic acid was used as the standard, and results were expressed as milligram of gallic acid equivalents (mg GAE/100 g) (Baiano et al., 2009).

2.5.2. Total flavonoid content (TFC)

Total flavonoid content was determined by the $AlCl_3$ colorimetric method (Chang et al., 2002). A 500 μL aliquot of appropriately diluted sample was mixed with1.5 mL methanol, 0.1 mL of a 10% $AlCl_3$ solution, 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water. After 30 min incubation at room temperature, the absorbance was measured at 415 nm versus a prepared water blank using a Jasco V-630 UV–vis spectrophotometer (Tokyo, Japan). Quercetin was used as a reference standard and the total flavonoid content was expressed as milligram of quercetin equivalent (mg QE/100 g).

2.5.3. HPLC-PDA-MS analysis

The chromatographic separation and mass spectrometric analyses of phenolics contained in the methanolic extracts were carried out on an Agilent 1100 series HPLC systems equipped with a photodiode array detector (PDA) and a triple quadrupole mass spectrometer type Micromass Autospec Ultima Pt interfaced with an ESI ion source. Separation was achieved using a Superspher®100 (12.5 cm × 2 mm i.d, 4 μm, Agilent Technologies, Rising Sun, MD) at 40 °C. The samples (20 μL) were eluted through the column with a gradient mobile phase consisting of A (0.1% acetic acid) and B (acetonitrile) with a flow rate of 0.25 mL/min. The following multi-step linear solvent gradient was employed: 0–5 min, 2% B, 5–60 min, 40% B, 60–85 min, 100% B, 85–85.1 min, 2% B, and 85.1–100 min, 2% B.

PDA detection was performed in the 250–550 nm wavelength range and the mass spectra were recorded in positive ion mode, under the following operating conditions: capillary voltage, 3.2 kV; cone voltage, 40 V; probe temperature, 350 °C; ion source temperature, 120 °C. The spectra were acquired in the m/z range of 150–750 amu.

Tentative identification of phenolic compounds was based on co-chromatography with authentic standards when available. PDA spectra and mass spectra were used to confirm the identity of compounds previously reported in the literature (Hanhineva et al., 2008; Cheng et al., 2009; Lin and Harnly, 2010; Ferreres et al., 2012).

2.6. Antioxidant activity

2.6.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The radical scavenging activity of the extracts against DPPH free radical was measured using the method of Brand-Williams et al. (1995), slightly modified as follows: an aliquot (1 mL) of methanol solution containing different amounts of each extract (0.01–1 mg/mL) was added to 2 mL of daily prepared methanol DPPH solution (0.1 mM). The mixture was shaken gently and left to stand at room temperature in the dark for 15 min. Thereafter, the absorbance was measured at 515 nm. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. BHT and trolox were used as positive controls. Results were expressed as radical scavenging activity percentage (%) of the DPPH according to the formula:

$$\%\ \text{DPPH radical scavenging} = [(A_0 - As)/A_0] * 100$$

where A_0 is the absorbance of the control and As is the absorbance of the sample. The effective concentration having 50% radical inhibition activity (EC_{50}) expressed as mg extract/mL was determined from the graph of the free radical scavenging activity (%) against the extract concentration. The antioxidant capacity of test samples was expressed as EC_{50}, the concentration necessary to 50% reduction of DPPH (Yuan et al., 2005).

2.6.2. ABTS (azinobis(ethylbenzothialzoline 6-sulphonic acid)) assay

The total antioxidant activity of the samples was measured by ABTS radicals according to the method of Re et al. (1999). Briefly, the radical cation $ABTS^+$ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. The blue-green ABTS solution was diluted in ethanol to give an absorbance of 0.7 at 734 nm prior to assay. The diluted ABTS solution (2850 μL) was mixed with 150 μL of sample extracts or trolox standard. The mixture was left to stand at room temperature in the dark for 15 min, and then the absorbance was measured at 734 nm. Trolox was used as positive control. The antioxidant capacity of test samples was expressed as EC_{50}, the concentration necessary to 50% reduction of $ABTS^+$ (Yuan et al., 2005).

2.6.3. 2-Deoxyribose assay

The 2-deoxyribose method was used for the determination of the scavenging effects of the methanolic extracts on hydroxyl radicals (Mimica-Dukic et al., 2003). The test sample of different concentrations was added to the reaction mixture containing 3 mM 2-deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM L-ascorbic acid and 1 mM H_2O_2. Phosphate buffer (20 mM, pH 7.4) was added up to a final volume of 4 mL. The mixture was incubated for 1 h at 37 °C, then 1 mL of 1% (w/v) thiobarbituric acid (TBA) and 1 mL of 2.8% (w/v) of trichloroacetic acid (TCA) were added in each mixture and heated for 30 min at 95 °C. After cooling at room temperature, the absorbance was measured at 532 nm against a blank containing 2-deoxyribose and buffer. BHT was used as positive control. Percentage inhibition of 2-deoxyribose degradation was calculated as follows:

$$I\,(\%) = [(A_0 - As)/A_0] * 100$$

where all symbols have the same meaning as in DPPH assay.

2.6.4. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Benzie and Strain (1996) with slight modifications. Stock solutions of 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine in 40 mM HCl), and 20 mM $FeCl_3$. $6H_2O$ were prepared. The working FRAP reagent was prepared by mixing the stock solutions in a 10:1:1 ratio. The solution was maintained at 37 °C and pH 3.6. Then, 150 μL of the sample was mixed with 2850 μL of the working solution and left standing at room temperature for 30 min in the dark. Absorbance of the mixture was then measured spectrophotometrically at 593 nm. The change in absorbance was calculated and related to the standard curve generated with Trolox. Results were expressed as micromole of Trolox equivalent per gram of extract (μmol TE/g).

2.7. Antimicrobial activity

2.7.1. Microbial strains and growth conditions

The yeast *Candida albicans* (ATCC 10231) and 7 bacterial strains including the Gram-positive *Staphylococcus aureus* ATCC 6538, *Enterococcus feacium* ATCC 19434, *Streptococcus agalactiae*, *Bacillus subtilis*, and the Gram-negative *Escherichia coli* ATCC 8739, *Enterococcus feacalis* ATCC 292129 and *Salmonella typhimurium* (ATCC 14028) were used to assess the antimicrobial properties of the extracts. *S. agalactiae* and *B. subtilis* were isolated in the Institut National des Sciences Appliquées (Tunis, Tunisia), while the remaining strains were obtained from the Institut Pasteur (Paris, France). Bacterial strains were cultured overnight at 37 °C in Mueller-Hinton (MH) agar.

2.7.2. Disk diffusion method

For the determination of the antimicrobial activity, the disk diffusion method was used according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997). Briefly, a suspension of the tested organism (10^8 CFU/mL) was spread on the MH solid media plate. Filter paper disk (6 mm in diameter) was soaked with 15 μL of the methanol extracts (dissolved in dimethyl sulfoxide) and placed on the inoculated plates. After being kept at 4 °C for 2 h, they were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeast. The definite zone of inhibition of any dimension surrounding the paper disk was measured accurately.

2.8. Phytotoxic activity

The phytotoxic effects of the methanolic extracts were evaluated using wild *Daucus carota*. Seeds of target species were surface sterilized with sodium hypochlorite 15% for 10 min and then rinsed with distilled water (Zahed et al., 2010). Twenty seeds were uniformly placed in a 9 cm sterile Petri dish lined with one layer of filter paper and moistened with 2 mL of *A. altissima* extracts at different concentrations or with 2 mL distilled water (control sample). Petri dish were then sealed with Parafilm (Neenah, Wisconsin, USA) to prevent evaporation and placed in germination cabinet at 22 °C in the dark. Germinated seeds with >1 mm radicle were recorded and radicle elongation was measured after 7 days of incubation.

2.9. Statistical analysis

All measurements were carried out in triplicate and the results were presented as mean values ± SD. Statistical analyses were performed using a one-way analysis of variance ANOVA test and the significance of the difference between means was determined by Duncan's multiple range test. Differences at $P < 0.05$ were considered statistically significant. The SPSS 13.0 (Chicago, Illinois, USA) was used to perform statistical analysis.

3. Results and discussion

3.1. Essential oil composition

The essential oil composition of *A. altissima*, along with the retention index, quantitative data and the identification methods, are listed in Table 1. A total of 139 compounds, covering more than 93% of the GC profiles were identified.

Comparison of the analytical data revealed marked differences in qualitative and quantitative compositions. Considering the main chemical classes, the three oil samples were characterized by their high content of non terpenic compounds (20.58–45.38%), sesquiterpene hydrocarbons (19.42–36.20%) and oxygenated monoterpenes (13.47–24.92%). The major constituents were tetradecanol, heneicosane, tricosane, docosane, α-curcumene, α-gurjunene, methyl decanoate, α-terpinen-7-al, geranial, α-guaiene, α-humulene and (*E*)-β-farnesene. The four former components were particularly abundant in the oil from population of Jrissa, while the remaining components were the most prominent ones in the oils from Bab Saâdoun and Bousalem populations. The latter which displayed the most complex composition, was also characterized by its relatively high amount of α-terpinene (4.26%) and decanal (2.56%). The observed compositional trend might be ascribed, at least partially, to differences in the genetic background and environmental factors. It is also logical to suggest that numerous metabolic pathways were evolved in the secondary metabolism of *A. altissima*.

When compared with the only publication regarding the essential oil composition of *A. altissima* (Mastelić and Jerković, 2001), our

Table 1
Chemical composition (% total peak area) of the essential oil of *A. altissima* leaves.

Compounds	RI	Jrissa	Bab Saâdoun	Bousalem
Octane	802	–	–	0.06
Isopropyl butanoate	830	–	–	0.06
2-Methyl-1-butanol	845	–	–	0.05
Nonane	900	–	–	tr
2-Heptanol	906	–	–	0.07
4-Methyl-3-thiazoline	917	–	0.41	0.06
α-Thujene	926	0.10	0.45	tr
α-Pinene	932	0.24	0.40	0.39
2-Pentanol	935	–	0.18	0.09
Fenchene	938	–	0.10	tr
Camphene	950	–	0.23	tr
(*E*)-2-heptenal	955	–	–	0.18
1-Octen-3-ol	963	–	0.83	0.52
Sabinene	970	–	0.86	0.21
β-Pinene	981	0.11	2.05	0.34
β-Myrcene	991	1.11	1.05	0.57
4-Carene	997	–	–	tr
α-Terpinene	1006	0.83	1.51	4.26
p-Cymene	1009	0.16	1.49	1.09
Limonene	1017	–	0.10	0.12
1,8-Cineole	1020	0.14	0.10	tr
Z-β-Ocimene	1026	–	–	tr
(E)-2-Octenal	1033	0.16	0.73	0.24
(E)-β-ocimene	1036	–	–	0.04
γ-Terpinene	1047	–	–	0.10
trans-Sabinene hydrate	1052	–	–	0.08
Nonen-3-one	1055	–	0.21	0.13
Camphenilone	1057	–	0.53	0.36
1-Octanol	1069	0.10	0.14	0.54
Nonan-2-one	1075	–	–	0.07
Terpinolene	1078	–	0.79	0.78
Nonanal	1082	0.23	0.23	0.78
Linalool	1085	–	0.76	0.69
α-Thujone	1095	–	–	0.24
Undecane	1101	0.78	0.21	0.31
3-Octanyl-acetate	1113	–	0.13	0.06
Limonene oxide	1115	–	–	tr
allo-Ocimene	1118	–	–	0.16
Camphor	1121	–	–	0.14
trans-para-Menth-2-ene-1-ol	1124	–	0.49	0.07
cis-Verbenol	1127	0.14	0.59	0.30
Pinocarvone	1139	–	0.12	0.06
cis-Chrysanthemyl alcool	1144	–	0.13	0.20
Lavandulol	1150	0.25	–	0.16
Borneol	1155	0.24	–	0.12
Cryptone	1159	–	–	0.05
Terpinen-4-ol	1163	1.61	0.42	1.17
p-Cymen-8-ol	1165	–	1.73	1.21
Estragol	1175	–	–	0.48
α-Terpineol	1176	0.36	–	0.11
Myrtenol	1178	–	2.38	0.89
Verbenone	1183	–	1.07	1.57
Decanal	1190	–	–	2.56
β-Cyclocitral	1198	0.36	2.00	1.31
Dodecane	1201	1.94	2.99	2.76
Nerol	1214	0.16	0.08	0.09
Hexyl-2-methyl butyrate	1223	–	3.11	1.57
Carvacryl methyl oxide	1226	–	1.19	0.46
Geraniol	1231	1.09	0.41	1.67
Linalyl acetate	1240	–	–	0.12
Geranial	1249	1.13	1.99	3.67
α-Terpinen-7-al	1254	**3.06**	**3.81**	**3.71**
Benzoïc acid	1259	–	0.24	0.08
Thymol	1263	–	0.42	0.45
Decanol	1266	–	–	tr
Bornyl acetate	1272	0.36	0.29	0.59
Carvacrol	1278	–	0.20	0.05
Undecanal	1280	–	–	0.21
(*E.E*)-2,4-decadienal	1287	–	–	0.09
Tridecane	1301	–	–	2.05
Methyl-decanoate	1308	2.92	1.51	4.21
Piperitenone	1318	1.10	0.62	0.90
Butyl benzoate	1324	–	0.16	0.22
2-Undecenal	1336	0.30	–	0.38
Neryl acetate	1344	–	–	0.21
α-Cubebene	1354	–	0.31	0.44
(E)-β-damascenone	1361	2.45	0.38	2.73
Geranyl acetate	1368	0.88	2.42	1.60
β-Boubonene	1383	0.14	1.05	1.19
(*E*)-β-damascone	1397	–	0.08	0.18
β-isocomene	1402	0.28	0.38	0.82
α-Cedrene	1407	0.48	0.14	1.13
α-Gurjunene	1414	**3.36**	**3.89**	**3.78**
β-Caryophyllene	1418	0.81	2.14	1.37
β-Copaene	1425	–	0.26	0.54
γ-Elemene	1430	0.14	0.17	0.40
Aromadendrene	1435	0.62	1.38	2.15
α-Guaiene	1438	1.47	1.74	1.77
(*E*)-β-farnesene	1446	1.36	2.49	3.16
α-Humulene	1457	1.04	1.79	4.47
allo-Aromadendrene	1465	0.94	2.39	2.57
α-Curcumene	1469	**4.00**	**6.86**	**5.92**
Germacrene-D	1474	0.24	–	0.26
trans-β-Bergamotene	1480	–	0.11	0.20
4-*epi*-Cubenol	1483	0.36	0.29	0.38
Valencene	1490	0.38	0.37	0.57
β-Bisabolene	1503	0.30	0.21	0.93
γ-Cadinene	1507	–	0.20	0.39
Cubebol	1514	–	1.12	0.77
δ-Cadinene	1518	1.63	–	1.15
Elemicine	1522	0.11	–	0.37
α-Bisabolene	1526	–	0.91	0.75
α-Cadinene	1534	0.32	0.46	0.59
α-Calacorene	1540	–	0.26	0.16
β-Calacorene	1546	0.12	–	0.32
Germacrene-B	1556	0.83	0.34	0.71
Spathulenol	1566	–	0.08	0.14
Caryophyllene oxide	1570	–	–	0.19
Globulol	1579	0.44	0.23	0.25
Viridiflorol	1584	–	0.14	tr
Ledol	1591	–	0.08	0.21
Humulene-6,7-epoxide	1596	–	0.29	0.18
γ-Eudesmol	1620	–	0.11	0.07
β-Eudesmol	1637	–	0.13	0.08
β-Acorenol	1655	0.13	–	0.06
Cadalene	1660	–	–	0.06
α-Bisabolol	1665	0.52	0.40	0.40
(E.Z)-Farnesol	1679	–	–	tr
Pentadecan-2-one	1686	–	–	0.13
Heptadecane	1700	–	0.09	tr
Mintsulfide	1728	0.64	0.12	0.15
β-Chamigrene	1741	–	–	tr
Tetradecanoïc acid	1752	–	–	tr
Hexadecanal	1791	0.25	0.40	0.17
Farnesyl acetate	1828	–	–	0.26
Pentadecanoïc acid	1870	0.35	0.26	0.49
iso-Eugenol	1884	0.53	0.32	0.16
Nonadecane	1900	0.32	0.53	0.11
hexadecanoïc acid	1957	0.85	0.49	0.29
Ethyl hexadecanoate	1973	–	–	tr
Manoyl oxide	1995	–	–	tr
Geranyl linalol	2031	–	–	0.05
α-Kaurene	2051	0.51	3.13	0.27
Tetradecanol	2093	**10.65**	0.09	0.10
Heneicosane	2100	**10.65**	0.16	–
(*E*)-phytol	2116	1.86	0.21	0.88
Dodecanoïc acid	2137	1.45	0.24	0.20
Docosane	2202	**5.88**	**11.77**	**0.96**
Tricosane	2302	**8.36**	–	0.61
Monoterpenes hydrocarbons		2.56	9.02	8.25
Oxygenated monoterpenes		13.47	22.21	24.92
Sesquiterpene hydrocarbons		19.42	30.97	36.20
Oxygenated sesquiterpenes		1.62	2.88	3.01
Diterpenes		1.86	0.21	0.91
Others		45.38	27.65	20.58
Total		84.31	92.95	93.87

RI: Retention indices on the apolar Rtx-1 column; values in bold indicate the main components; tr: trace (<0.05%); –: not detected.

results displayed striking qualitative and quantitative differences. In the aforementioned study, the chemical composition of the leaf oil was found to be rich in aliphatic C_6-compounds ((*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenyl butanoate and (*E*)-2-hexenal, among others), sesquiterpene hydrocarbons (β-caryophyllene, γ-cadinene and calarene) and oxygenated monoterpenes namely linalool and β-cyclocitral. They also found that the chemical composition was greatly influenced by the plant developmental stage and the sample preparation.

In another report, the chemical composition of the essential oil of the closely related species; *A. excelsa* was described (Tzakou et al., 2006). The main components were found to be phytol, linolenic acid, linoleic acid and palmitic acid. At this point, it can be presumed that the chemical polymorphism of the essential oils of *Ailanthus* sp. substantially depends upon the species, plant part, developmental stage, sample preparation and analysis.

3.2. Contents of total phenolics and flavonoids

As shown in Table 2, plants from Bousalem had a significant ($p < 0.05$) higher content of total phenolics (119.84 mg GAE/g dry extract) in comparison with those from Jrissa and Bab Saâdoun (31.53 and 33.13 mg GAE/g dry extract, respectively). The same trend was also observed in the total phenolic contents in the residual leaves (37.95, 16.37 and 5.85 mg GAE/g dry extract in Bousalem, Bab Saâdoun and Jrissa, respectively). In contrast, plants from Jrissa contained the highest contents of total flavonoids in intact leaves (1.75 mg QE/g dry extract) and in residual leaves (77.24 mg QE/g dry extract). At this point, it seems that heat treatment (during hydrodistillation) associated with increased pressure enhance the disintegration of cell structure which resulted in the increased release of cell contents (including flavonoids) into bulk medium. Support to this assumption is given by Kima et al. (2012), who reported that thermal treatment (100 °C) of black garlic induced an increase in the total flavonoid content. Similarly, Chen and Chen (2011) found increased total flavonoid contents in sumac fruits by using steam explosion procedure at 200 °C. They argued that the improved recovery of flavonoids from heat treated sample was mainly due to breakage and destructuration of cell walls, leading to enhanced release of these metabolites. In the same way, Hartonen et al. (2007) have reported that flavonoids from *Populus tremula* were most efficiently extracted with pressurized hot water extraction (PHWE) at 150 °C. When compared with earlier works, the values obtained were lower than those reported by Luís et al. (2012) for the same species. According to these authors, the content of total phenolics and flavonoids varied depending on plant part and the solvent of extraction.

3.3. Characterization of phenolic compounds by HPLC-DAD–MS

The typical phenolic profile of *A. altissima* sample is depicted in Fig. 1. Data of the retention time (*tr*), UV, mass spectra and tentative identification are presented in Table 3. As can be seen, 9 components were characterized and 5 of them (peaks **1**, **2**, **4**, **5** and **7**) were unambiguously identified by comparing their retention time, UV and MS spectra with those of reference standards. Peak 1 ($tr = 3.35$ min, λmax: 269 nm) exhibited $[M–H]^-$ base ion at m/z 169. By comparing its *tr*, UV spectrum, molecular mass and fragmentation pattern with the reference standard, this compound was identified as gallic acid. Peak 2 ($tr = 17.78$ min, λmax = 323 nm) exhibited $[M–H]^-$ base ion at m/z 353 and fragmentation at m/z 191 [quinic acid–H]$^-$. The *tr*, UV spectrum and MS data were identical to those of the reference standard of chlorogenic acid (5-cafeoylquinic acid). So it was identified as chlorogenic acid. Peak 4 ($tr = 24.55$ min; λmax: 277 nm) was identified as epicatechin based on its retention time, UV absorbance and spectral data of the reference standard epicatechin ($[M–H]^-$ at *m/z* 289).

Peak 5 ($tr = 28.48$ min; λmax: 260–359 nm) showed a $[M–H]^-$ ion at m/z 609 with a base peak at m/z 301 indicative of the quercetin aglycone. This compound was assigned as rutin (quercetin-3-*O*-rutinoside) by comparison of retention time, UV spectrum and mass spectrometric data with rutin reference standard. Peak 7 ($tr = 31.19$ min; λmax: 257, 353 nm) showed a $[M–H]^-$ ion at m/z 463 and a fragment $[M–H-162]^-$ ion at m/z 301 (quercetin aglycone) due to the elimination of hexose unit (162 amu). This compound was identified as quercetin-3-*O*-galactoside (hyperoside) by comparison of the retention time, UV and mass spectra with authentic standard.

The remaining components were provisionally identified based on the analysis of their UV, mass spectra and comparison with literature data (Barry et al., 2001; Hanhineva et al., 2008; Cheng et al., 2009; Ferreres et al., 2012). Accordingly, peak 3 ($tr = 22.77$ min; λmax: 265 nm) displayed a pseudo molecular ion $[M–H]^-$ at *m/z* 633 with a base peak at *m/z* 463 and at *m/z* 301 corresponding to loss of gallic acid (170 amu) and HHDP (302 amu), respectively. This compound was tentatively identified as HHDP-galloylglucose (Barry et al., 2001; Hanhineva et al., 2008). Peak 6 ($tr = 29.77$ min; λmax: 271, 367 nm) exhibited pseudomolecular ion $[M–H]^-$ at *m/z* 615 and major fragment ions at *m/z* 463 ($[M–H-152]^-$) and *m/z* 301 ($[M–H-152–162]^-$) corresponding to losses of galloyl unit (152 amu) and hexose unit (162 amu), respectively. By comparison with the spectral data of Hanhineva et al. (2008), Cheng et al. (2009) and Ferreres et al. (2012), this component was tentatively identified as quercetin-3-galloyl hexoside. Peak 8 ($tr = 34.22$ min) exhibited a $[M–H]^-$ ion at *m/z* 447 and two absorption maxima at 263 and 349 nm, indicative of luteolin glucoside (Lin and Harnly, 2010; Lesh and Jarosz, 2011), but they were not informative enough to identify this compound.

Peak 9 ($tr = 34.90$ min) showed a $[M–H]^-$ ion at *m/z* 533 and major fragment at *m/z* 489 $[M–H– CO_2]^-$ (corresponding to loss of 44 amu). The UV spectrum showed two absorption maxima at 267 and 335 nm, which are characteristics of an apigenin derivative (Boué et al., 2003; Hong et al., 2006). The observed fragmentation pattern and the UV spectrum, however, are not sufficient to elucidate the structure of this component.

Previous studies regarding the phenolic constituents of *Ailanthus* plants have led to the identification of 19 flavonoids (Kundu and Laskar, 2010). According to El-Baky et al. (2000), quercetin, kaempferol, isoquercetin, kaempferol-3-*O*-glucoside, rutin and luteolin-7-*O*-β-(6″ galloylglucopyranoside) were the main representative flavonoids in *A. altissima* leaves. In a recent report from Portugal, the phenolic profile of *A. altissima* was found to be rich in phenolic acids namely ferulic, ellagic, chlorogenic, *p*-coumaric, syringic, caffeic and vanillic acids (Luís et al., 2012). Loizzo et al. (2007) studied the phenolic composition of *A. excelsa* leaves and found that apigenin, luteolin, kaempferol-3-*O*-α-

Table 2
Total phenolics and flavonoid contents in *A. altissima* leaves and their hydrodistilled residues.

	Total phenols (mg GAE/g dry extract)		Total flavonoids (mg QE/g dry extract)	
	Leaves	Leaf hydrodistilled residues	Leaves	Leaf hydrodistilled residues
Jrissa	31.53 ± 0.44^b	16.37 ± 1.27^c	1.75 ± 0.03^a	77.24 ± 1.47^a
Bab Saâdoun	33.13 ± 1.87^b	5.85 ± 0.48^b	0.92 ± 0.15^b	75.57 ± 3.4^a
Bousalem	119.84 ± 1.47^a	37.95 ± 0.52^a	0.97 ± 0.09^b	39.49 ± 0.72^b

Values given are mean ± standard deviation of triplicate samples; superscripts with different letters within the same column are significantly ($p < 0.05$) different.

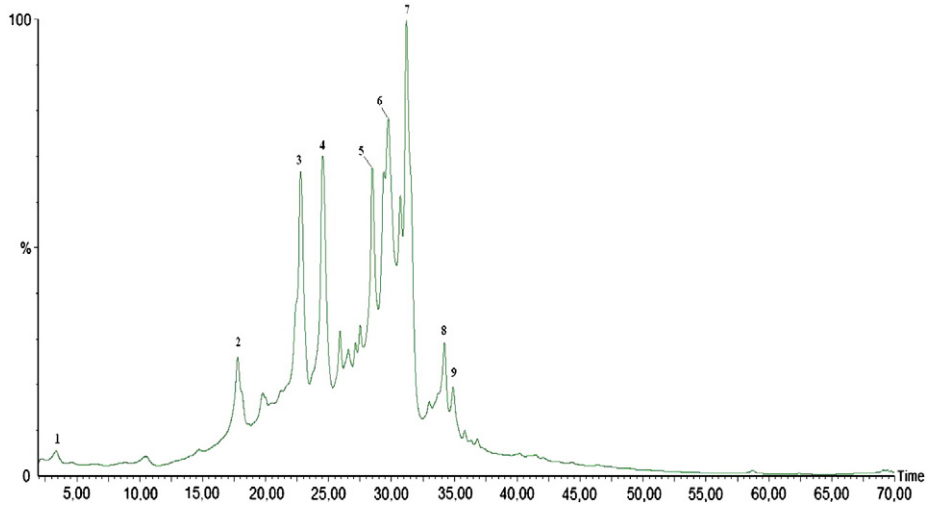

Fig. 1. Representative LCMS–TIC of phenolic components in leaf methanolic extract of *A. altissima*.

arabinopyranoside, kaempferol-3-*O*-β-galactopyranoside, quercetin-3-*O*-α-arabinopyranoside and luteolin-7-*O*-β-glucopyranoside were the main flavonoids. When compared with the aforementioned compositional studies, our results displayed striking differences presumably due to the origin, species and analytical conditions. Nevertheless, knowledge of *Ailantus* phenolic compounds was extended by identifying 4 compounds (hyperoside, epicatechin, quercetin-3-galloyl hexoside and HHDP galloylglucose) not previously reported in this plant.

From this result, it emerges that *A. altissima* might be considered as consolidated source of bioactive components justifying hence its medicinal properties. Phenolic compounds such as gallic acid, chlorogenic acid, quercetin, rutin and epicatechin, among others, are well known antioxidants which can provide relief from certain physical ailments and degenerative diseases including the reduction of cardiovascular disease and certain cancers (Craft et al., 2012). Bearing this in mind, the antioxidant activity of methanolic extracts prepared from *A. altissima* leaves and their hydrodistilled residues was determined by four complementary test systems: DPPH, hydroxyl radical (2-deoxyribose), FRAP and reducing power.

3.4. Antioxidant activity

3.4.1. DPPH scavenging activity

DPPH has been extensively used as a valid, easy and inexpensive assay to evaluate radical scavenging ability of antioxidant. As shown in Fig. 2, all methanolic extracts showed a concentration-dependent scavenging activity. Leaf methanolic extract from Bousalem proved to be the strongest DPPH scavenger with a mean EC_{50} of 14.77 μg/mL followed by Bab Saâdoun (81.39 μg/mL) and Jrissa (86.61 μg/mL). The methanolic extracts of the hydrodistilled residues showed a similar inhibitory effect (Table 4). The DPPH scavenging activity of samples from Bousalem (EC_{50} of 56.82 μg/mL) was 3 and 13-fold higher than those of Jrissa and Bab Saâdoun, respectively. These results reflect the hydrogen-donating ability of the extracts from leaves and their residues. On the other hand, the higher DPPH scavenging activity of samples from Bousalem is most likely attributed to their higher total phenolic contents.

3.4.2. ABTS scavenging activity

The ABTS scavenging assay, which employs a specific absorbance (734 nm) at a wavelength remote from the visible domain, is rapid, sensitive, and reproducible, and requires simple laboratory equipment (Du et al., 2009) can be used in both organic and aqueous system and can also be an excellent index reflecting the antioxidant activity of the test samples (Wang et al., 2010). Fig. 3 illustrates the concentration-dependent scavenging activity of the methanolic extracts from *A. altissima* leaves and their hydrodistilled residues. The scavenging activity of samples from Bousalem were significantly ($p < 0.05$) higher (EC_{50} of 8.64 and 198.98 μg/mL for leaves and their hydrodistilled residues, respectively) than those from Jrissa and Bab Saâdoun (Table 4).

3.4.3. Hydroxyl radical scavenging activity

The ability of *A. altissima* leaves and their hydrodistilled residues to scavenge hydroxyl radicals generated by Fenton reaction was evaluated by hydroxyl radical-induced deoxyribose degradation. There is a concentration-dependent inhibition of hydroxyl radical by all extracts of *A. altissima* (Fig. 4). Table 4 shows that leaf methanolic extracts from Jrissa exhibited lower (EC_{50} 5 μg/mL) but not significant ($p < 0.05$) hydroxyl radical scavenging activity than those from Bab Saâdoun (EC_{50} 4.63 μg/mL) and Bousalem (EC_{50} 4.42 μg/mL). Concerning the methanolic extracts of hydrodistilled leaves, the best results were observed with samples from Bab Saâdoun (EC_{50}

Table 3
Retention time, maximum UV absorption and deprotonated molecular mass of phenolic and flavonoid components identified in *A. altissima*.

Peak no.	RT (min)	UV (λmax)	$[M–H]^-$	Tentative identification
1	3.35	269 nm	169	Gallic acid
2	17.78	323 nm	353	Chlorogenic acid
3	22.77	265 nm	633	HHDP-galloylglucose
4	24.55	277 nm	289	Epicatechin
5	28.48	260, 359 nm	609	Rutin
6	29.77	271, 367 nm	615	Quercetin-3-galloyl hexoside
7	31.19	257, 353 nm	463	Quercetin-3-*O*-galactoside (hyperoside)
8	34.22	263, 349 nm	447	Luteolin glucoside
9	34.90	267, 335 nm	533	Apigenin derivative

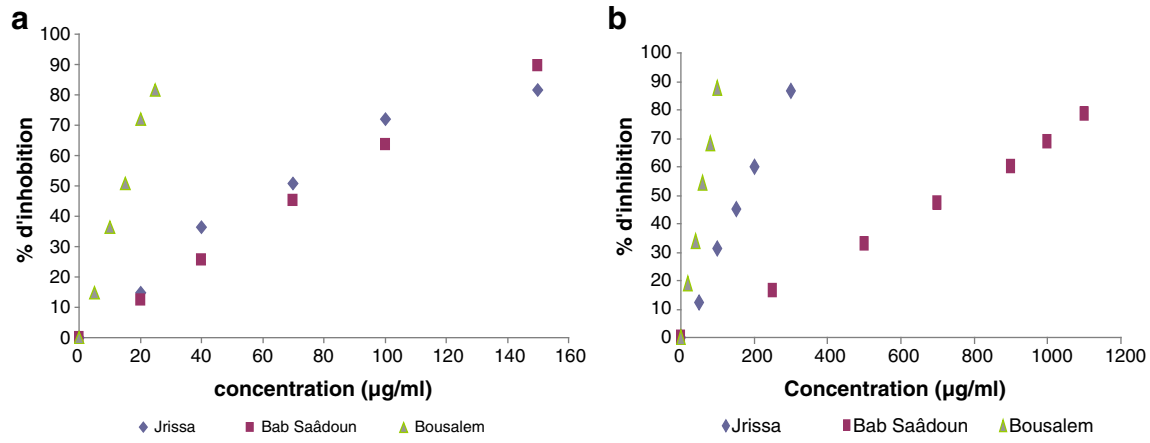

Fig. 2. Concentration-dependent scavenging activity of DPPH radicals of the methanolic extracts of *A. altissima* leaves (a) and their hydrodistilled residues (b).

3.82 µg/mL) followed by Jrissa (EC_{50} 4.44 µg/mL) and Bousalem (EC_{50} 4.99 µg/mL). In any case, the hydroxyl radical scavenging activity of all extracts was significantly higher than that of the standard antioxidant BHT where EC_{50} was achieved at 5.98 µg/mL.

3.4.4. Ferric reducing antioxidant power (FRAP)

The FRAP assay is based on the reduction of Fe(III)–tripyridyltriazine (Fe(III)–TPTZ) complex to Fe(II)–tripyridyltriazine (Fe(II)–TPTZ) at low pH by electron-donating antioxidants, resulting in the absorbance increase at 593 nm (Raudonis et al., 2012). As displayed in Table 4, the FRAP values of leaf methanolic extracts from Jrissa (334 µmol TE/g) and Bousalem (327.5 µmol TE/g) were significantly ($p < 0.05$) higher than that of Bab Saâdoun (242.5 µmol TE/g). The methanolic extract from leaf hydrodistilled residues of Bousalem showed significantly higher ferric reducing activity (261 µmol TE/g) when compared with Bab Saâdoun (106.25 µmol TE/g) and Jrissa (87 µmol TE/g).

In general, the methanolic extracts of leaves and their hydrodistilled residues could be considered as effective scavengers of DPPH, ABTS and hydroxyl radicals as well as potent reducing agents. From a mechanistic standpoint, the observed antioxidant activity reflects the ability of the test extracts to donate electrons or hydrogen atoms to inactivate radical species (Yuan et al., 2005). Such properties have been reported for numerous phenolic compounds namely gallic acid (Lu et al., 2006), chlorogenic acid (Sato et al., 2011), rutin (Yang et al., 2008), hyperoside (Piao et al., 2008), HHDP galloylglucose (Tan et al., 2012), epicatechin (Iacopini et al., 2008), luteolin glucoside (Jeong et al., 2010) and apigenin derivative (Siger et al., 2012). Additionally, these results confirm the traditional and therapeutic uses including antiproliferative, anti-inflammatory and the treatment of nervous and cardiovascular disease of *A. altissima* (Kundu and Laskar, 2010). On the other hand, they also support the analytical HPLC data. Overall, our results are in good agreement with those of Rahman et al. (2009), although that these authors were limited to only DPPH assay in their evaluation of the antioxidant activity of methanolic extracts from *A. altissima* leaves. By using two in vitro tests (DPPH and β-carotene bleaching test), similar results have also been reported by Luís et al. (2012).

3.5. Antimicrobial activity

The methanolic extracts of *A. altissima* leaves and their hydrodistilled residues were screened for their antimicrobial activity against 4 Gram-positive bacteria, 3 Gram-negative bacteria and one yeast. The results in terms of zone of inhibition (mm) showed that leaf methanolic extracts were effective against Gram-positive bacteria (*S. aureus*, *E. feacium*, *S. agalactiae* and *B. subtilus*). Neither the Gram-negative bacteria (*E. coli*, *E. feacalis* and *S. typhimurium*) nor the yeast (*C. albicans*) were inhibited by these extracts (Table 5). Among the test extracts, those from bousalem were found as the most efficient antibacterial. At this point, the antibacterial activity appears to correlate well with total polyphenol contents as previously reported in *Teucrium arduini* (Šamec et al., 2010). The methanolic extract of leaf hydrodistilled residues was found to be effective against two Gram-positive bacteria (*S. agalactiae* and *E. feacium*), while they were not active against the remaining strains. The resistance of Gram-positive bacteria towards plants extracts has been previously reported (Tian et al., 2009; Šamec et al., 2010; Hosni et al., 2013). At this point, the occurrence of a very restrictive outer membrane consisting of lipoproteins and lipopolysaccharides resulted in a high level of intrinsic resistance to active components rendering the Gram-negative bacteria less susceptible to plant extracts than the Gram-positive bacteria. From a fundamental point of view, the results observed

Table 4
DPPH, ABTS, hydroxyl radical scavenging activities and ferric reducing power of the methanolic extracts of leaves and their hydrodistilled residues.

	DPPH EC_{50}(µg/mL)	ABTS EC_{50}(µg/mL)	OH radical EC_{50}(µg/mL)	FRAP (µmol TE/g)
Leaves				
Jrissa	86.61 ± 1.27^{a}	20.25 ± 0.28^{a}	5.01 ± 0.41^{a}	334 ± 7.49^{a}
Bab Saâdoun	81.39 ± 0.81^{a}	21.66 ± 1.53^{a}	4.63 ± 0.21^{a}	242.5 ± 7.07^{b}
Bousalem	14.78 ± 0.24^{b}	8.64 ± 0.51^{b}	4.42 ± 0.1^{a}	327.5 ± 6.78^{a}
Leaf hydrodistilled residues				
Jrissa	174.06 ± 3.45^{B}	632.97 ± 8.01^{B}	4.45 ± 0.03^{B}	87 ± 3.53^{A}
Bab Saâdoun	731.74 ± 6.15^{A}	3263.88 ± 120.27^{A}	3.82 ± 0.24^{C}	21.77 ± 0.88^{B}
Bousalem	56.82 ± 2.47^{C}	198.98 ± 3.77^{C}	4.99 ± 0.31^{A}	4.97 ± 0.2^{C}
BHT EC_{50} (µg/mL)	19.24 ± 0.32		5.98 ± 0.22	
Trolox EC_{50} (µg/mL)	13.69 ± 0.04	64.37 ± 1.28		

Superscripts with different letters within the same column are significantly ($p < 0.05$) different.

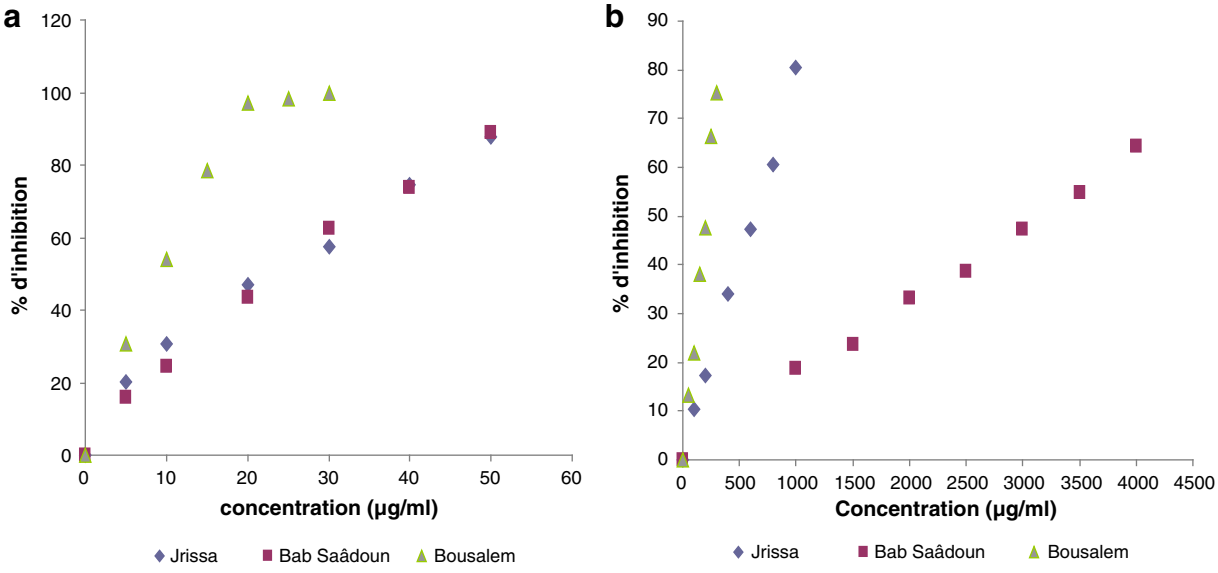

Fig. 3. Concentration-dependent scavenging activity of ABTS radicals of the methanolic extracts of *A. altissima* leaves (a) and their hydrodistilled residues (b).

might be attributable to the occurrence of some specific components such as gallic acid (Chanwitheesuk et al., 2007), rutin (China et al., 2012), hyperoside (Van der Watt and Pretorrius, 2001), epicatechin (Betts et al., 2011), luteoline derivatives and apigenin derivatives (Cushnie and Lamb, 2005). Moreover, the results obtained give a scientific support and confirm the traditional medicinal use of this species as wound healing (Kundu and Laskar, 2010). From practical standpoint, the results obtained encourage the use of species as food preservative and for pharmaceutical purposes.

In the specific case of *A. altissima*, data on its antimicrobial activity are very scanty. The only study by Rahman et al. (2009) reported that the leaf methanolic extracts of *A. altissima* were more effective against Gram-positive bacteria namely *Listeria monocytogenes*, *S. aureus*, *B. subtilis* and *Pseudomonas aeruginosa*. However, their extracts were ineffective against the Gram-negative bacteria such as *E. coli*, *Salmonella enteritidis* and *Salmonella typhimurium* which is in good agreement with our findings.

3.6. Phytotoxic effects

Tables 6 and 7 report the effects of leaf hydrodistilled residues of methanolic extracts on the germination and radicle growth of *D. carota*. The data clearly show that both extracts inhibit germination and radicle growth of the target species in a concentration-dependent manner. All the applied concentrations reduced the germination of the test weed. The inhibitory potential of the extracts, however, was found to vary with the origin of the plant material, with those from Bousalem being the most efficient.

Germination percentage was severely inhibited by the leaf hydrodistilled residues, where 400 to 600 µg/mL were sufficient to achieve complete inhibition of germination. Comparative analysis of the extracts showed that those from Bab Saâdoun were the more toxic. With regard to radicle growth, a similar trend was observed. From these results, the phytotoxic effect seemed to correlate better with the extracts of higher phenolic contents (Bousalem). Such results have previously been reported in *Psychotria leiocarpa* (Corrêa et al., 2008).

Additionally, the presence of some putative allelochemicals such as gallic acid (Xuan et al., 2005), chlorogenic acid (Chung et al., 2002), glucosylated quercetin (Macías et al., 2007) and glucosylated luteolin (Beninger and Hall, 2005) might explain the results observed. However, we cannot infer a reliable conclusion about the exact identity of the compounds responsible for the phytotoxic effects. Quassinoids such as ailanthone, ailanthinone, chapparines and

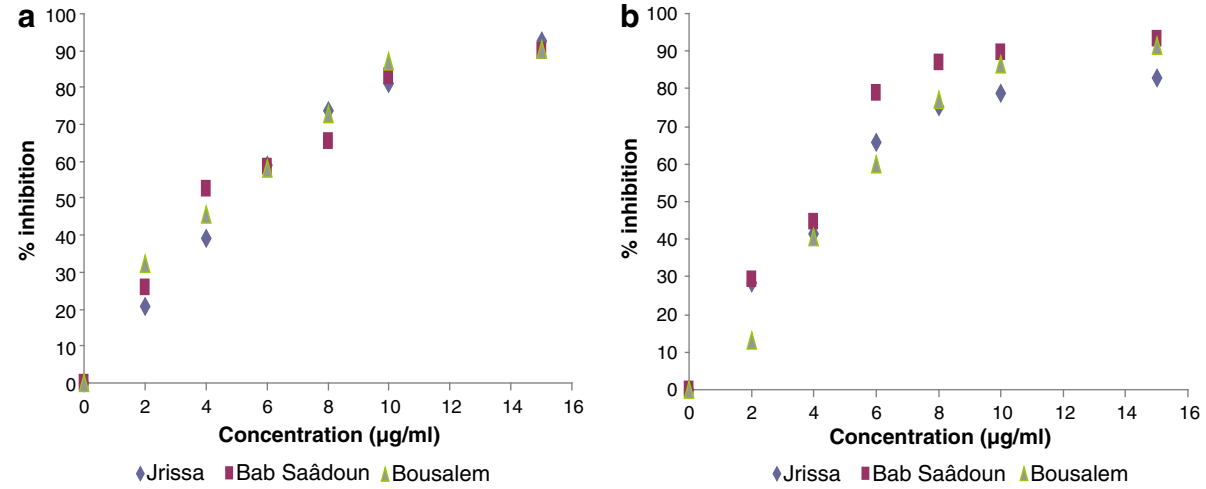

Fig. 4. Concentration-dependent scavenging activity of hydroxyl radicals of the methanolic extracts of *A. altissima* leaves (a) and their hydrodistilled residues (b).

Table 5
Antimicrobial activity (zone of inhibition mm) of the methanolic extracts of *A. altissima* leaves and their hydrodistilled residues.

	Leaves			Leaf hydrodistilled residues			Ampicillin
	Jrissa	Bab Saâdoun	Bousalem	Jrissa	Bab Saâdoun	Bousalem	
S. aureus (+)	14 ± 0.00	17.33 ± 0.65	23 ± 2.99	–	–	–	24.33 ± 1.24
E. feacium (+)	14.5 ± 3.53	15 ± 0.00	18 ± 4.24	–	–	–	44.66 ± 0.47
S. agalactiae (+)	23.66 ± 2.77	22 ± 1.41	16.5 ± 0.71	13 ± 1.41	11.33 ± 2.08	16 ± 2.94	42 ± 1.41
B. subtilus (+)	19.66 ± 2.51	18.33 ± 1.58	24.66 ± 2.51	–	–	–	43.33 ± 0.57
E. coli (−)	–	–	10 ± 2.42	–	–	–	19.5 ± 1.5
E. feacalis (−)	–	–	–	–	–	21.66 ± 2.35	45 ± 0.00
S. typhimurium (−)	–	–	–	–	–	–	26.5 ± 2.12
C. albicans (yeast)	–	–	–	–	–	–	22 ± 1.41

Values are mean ± standard deviation of triplicate measurements.

Table 6
Effects of methanolic extracts of *A. altissima* leaves and their hydrodistilled residues on the germination (%) of wild *Daucus carota*.

	Concentration (μg/mL)					
	0	50	100	200	400	600
Leaves						
Jrissa	100	53 ± 3	44 ± 5	41 ± 7	37 ± 5	26 ± 2
Bab Saâdoun	100	49 ± 4	47 ± 2	41 ± 3	29 ± 3	23 ± 2
Bousalem	100	49 ± 3	39 ± 5	35 ± 5	15 ± 2	6 ± 1
Leaf hydrodistilled residues						
Jrissa	100	41 ± 3	24 ± 3	15 ± 2	4 ± 1	0
Bab Saâdoun	100	38 ± 7	21 ± 2	4 ± 2	0	0
Bousalem	100	35 ± 2	32 ± 3	29 ± 6	8 ± 1	0

Values are mean ± standard deviation of triplicate measurements.

ailanthinol and alkaloids such as 1-methoxycanthin-6-one, frequently present in *A. altissima* may also have contributed to the phytotoxic effect observed (Lin et al., 1995). These components have previously been shown to act as allelopathic agents (De Feo et al., 2003). Research in this direction (characterization of quassinoids and alkaloids) is currently in progress in our laboratory.

The phytotoxic effect of *A. altissima* is unequivocally the best studied biological properties of this species (De Feo et al., 2003). These authors have reported that the aqueous root extracts of *A. altissima* exhibited a dose-dependent inhibition of the germination and radicle growth of radish (*Raphanus sativus* L. cv. "saxa"), garden cress (*Lepidium sativum* L.) and purslane (*Portulaca olearacea* L.). They also found that the main allelochemicals were ailanthone, ailanthinone, chaparrine and ailanthinol with the former component being the most powerful allelochemical. Eight years earlier, Lin et al. (1995) have identified ailanthinone as the active principle responsible for the inhibition of the growth of *Brassica juncea*, *Eragrostis tef* and *Lemna minor*. Under field conditions, Heisey and Heisey (2003) have tested the herbicidal effect of the methanol extracts from stem and bark of *A. altissima* on 17 weed and crop species and found that their application provided strong herbicidal effect on all tested species. Another report from USA showed that that *A. altissima* has important negative effects on seedling growth of dominant native tree species (Gómez-Aparicio and Canham, 2008). In a recent paper, Pedersini et al. (2011) have reported that the ailanthone-rich dichloromethane extract from the bark of *A. altissima* had potent pre-and post-emergence herbicidal activity on herbaceous species.

Overall, the present results represent further support to the phytotoxic effects of this species and explain its successful establishment in non-native range.

4. Conclusions

As evidenced by all the data above, we concluded that *A. altissima* leaves could be considered as a consolidated source of bioactive components. Methanolic extracts from leaves and their hydrodistilled residues possessed strong antioxidant and phytotoxic activities. They also exhibited antibacterial activity against Gram-positive bacterial strains. This data adds valuable information to the existing knowledge on *A. altissima*, as well as being useful in the formulation of supplementary food and a pharmaceutical. The development of a new natural herbicide would be another commercial use of this underutilized species.

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Table 7
Effects of methanolic extracts of *A. altissima* leaves and their hydrodistilled residues on radicle growth (mm) of wild *Daucus carota*.

	Concentration (μg/mL)					
	0	50	100	200	400	600
Leaves						
Jrissa	4.33 ± 1.2	1.97 ± 0.5	1.54 ± 0.71	1.27 ± 1.2	1.31 ± 0.9	1.12 ± 1.1
Bab Saâdoun	4.44 ± 1.8	2.31 ± 1.27	2.12 ± 0.93	1.75 ± 0.67	1.62 ± 0.6	1.18 ± 0.78
Bousalem	4.45 ± 0.7	4.32 ± 1.05	4.15 ± 1.74	3.95 ± 1.45	3.3 ± 1.07	2.1 ± 1.06
Leaf hydrodistilled residues						
Jrissa	4.11 ± 1.21	4.03 ± 1.45	3.48 ± 1.71	2.56 ± 0.51	0.92 ± 0.39	0
Bab Saâdoun	5.03 ± 2.13	4.42 ± 1.52	2.83 ± 0.68	2.5 ± 1.02	0	0
Bousalem	4.86 ± 1.22	3.68 ± 0.87	2.45 ± 1.24	2.26 ± 0.87	1.85 ± 0.39	0

Values are mean ± standard deviation of triplicate measurements.

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